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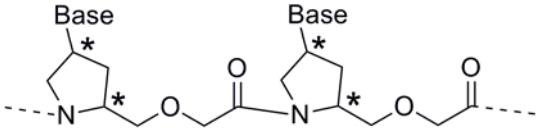

Pyrrolidine-based oxy-PNA (POPNA)
(Configuration; *trans*-L, *cis*-L, *trans*-D and *cis*-D, Base: A, T, G, and C)

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ABSTRACT

We synthesized 16 pyrrolidine-based oxy-peptide nucleic acid (POPNA) monomers carrying four different nucleobases onto four different stereoisomers of pyrrolidine rings. Using these monomers, we prepared POPNA oligomers, which formed sequence-specific hybrids with DNAs. The oligomer configurations influenced the hybrid stability. The oligomers were not taken into CHO cells. However, they could enter the cell cytoplasm when mixed with the influenza virus hemagglutinin peptide-arginine heptamer conjugate.

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1. Introduction

Nielsen-type peptide nucleic acids (PNAs; Figure 1 A) are DNA surrogates that form stable duplexes and triplexes with DNAs (Figure 1 B) and RNAs.[1] The non-ionic backbone of PNAs leads to stable hybrids with the nucleic acids, but at the same time, the neutral backbone results in low water solubility of the PNAs owing to the formation of aggregates in aqueous solutions. This undesirable property limits the use of PNAs in medicinal and other applications. One way of overcoming this drawback is to conjugate PNAs with cationic peptides such as cell-penetrating peptides (CPPs).[2] However, the PNA-CPP conjugates cause non-specific interactions with nucleic acids consisting of anionic backbones.

To overcome these drawbacks, we synthesized new peptide nucleic acids by introducing ether linkages in the main chain of peptide nucleic acids; An example is oxy-PNA (OPNA; Figure 1 C).[3] Introduction of ether linkages in the PNA backbones can improve the water solubility of the PNAs.[3a, 4] OPNAs are also not expected to non-specifically interact with nucleic acids because of the non-ionic backbone of the OPNAs. The OPNA oligomers can be successfully hybridized with the complementary DNAs, which is indicated by very sharp melting curves. However, because OPNAs do not form stable hybrids with RNAs, we designed and synthesized other types of oxy-PNAs that whose structures resembled the structure of DNA by introducing pyrrolidine rings in the backbone; An example is pyrrolidine-based oxy-PNAs (POPNAs; Figure 1 D).[5,6]

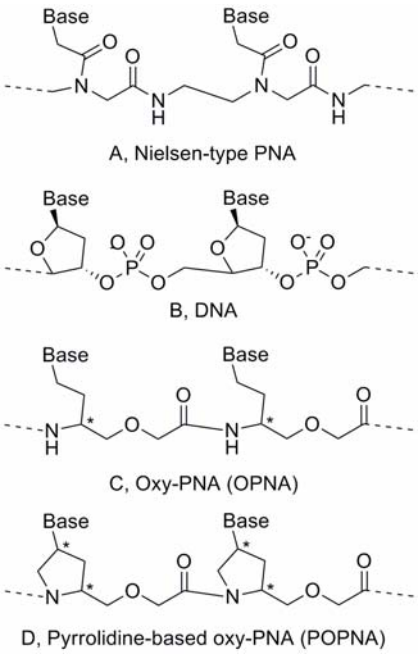


Figure 1. Chemical structures of PNA, DNA, OPNA, and POPNA oligomers.

The pyrrolidine ring possesses two chiral centers and there are four stereoisomers (*cis*-L-, *trans*-L-, *cis*-D-, and *trans*-D-configurations). We have previously shown that among the adenine homo-oligomers of the four different configurations, *trans*-L-POPNA and *cis*-L-POPNA form the most stable hybrids with the complementary RNA and DNA, respectively.[5d] In this study, we synthesized sixteen POPNA monomers of four stereoisomers on the pyrrolidine ring with four different nucleobases (adenine (A), thymine (T), guanine (G), and cytosine (C)). The coupling of these monomers resulted in the synthesis of POPNA oligomers that contained sequences of the four nucleobases. The coupling was performed by Fmoc-based solid-phase peptide synthesis (SPPS). The formation of hybrids of these POPNA oligomers with the DNA sequences was examined by studying the melting curves. We also investigated the cellular uptake and endosomal release of the POPNA oligomers. The cellular uptake of POPNA oligomers was achieved by mixing the POPNA oligomers with HA2 (an *N*-terminal 23-mer peptide of the influenza virus hemagglutinin protein)[7] conjugated with a CPP consisting of an arginine heptamer.[8]

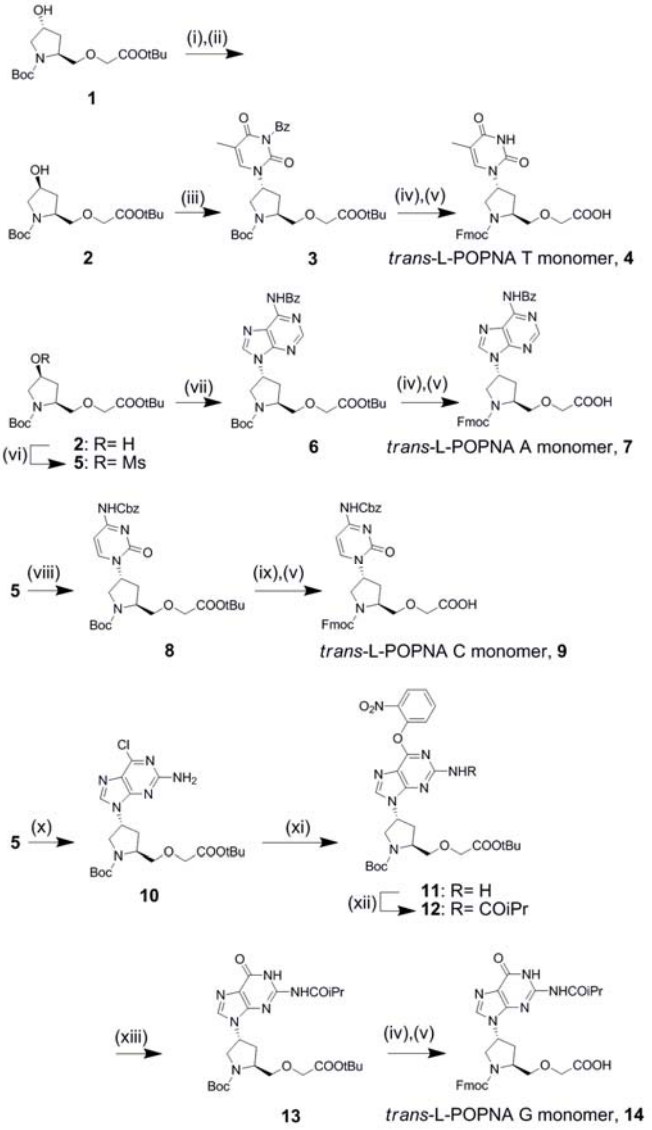
2. Results and discussion

2.1. Synthesis of POPNA monomers and their oligomers

Sixteen POPNA monomers were synthesized from two compounds, **1** (the *trans*-L-**1**, see Scheme 1) and the *cis*-D-**1**. These starting materials, **1** and the *cis*-D-**1**, were synthesized from *trans*-L-hydroxyproline and *cis*-D-hydroxyproline in 6 steps according to the literature procedure, respectively.[5b] *Cis*-L-**2** and *trans*-D-**2** were synthesized in two steps through the inversion of the free hydroxyl group—in the 4th position of the pyrrolidine ring of *trans*-L-**1** and *cis*-D-**1**—by formylation and subsequent treatment with aqueous ammonia in methanol. The synthetic routes from the compound **2** to the *trans*-L-POPNA monomers with A, T, G, and C nucleobases are shown in Scheme 1.

For preparing the *trans*-L-POPNA T monomer, first, compound **3** was synthesized by a direct substitution of the hydroxyl group of **2** with N^3-benzoylthymine[9] under Mitsunobu conditions.[5,10] Next, **3** was treated with 30% HBr/AcOH to remove the *tert*-butyloxycarbonyl (Boc), *tert*-butyl ester (tBu), and benzoyl (Bz) protecting groups. The resulting free amine was then protected with 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu) to give the *trans*-L-POPNA T monomer **4** in an overall yield of 85% from **2**. For the *trans*-L-POPNA A monomer, the hydroxyl group of **2** was first reacted with methanesulfonyl chloride (Ms-Cl) to give the mesylate **5** in 90% yield. Next, **5** was alkylated with N^6-benzoyladenine to give compound **6** in 55% yield.[11] Removal of Boc, tBu, and Bz protecting groups on **6** with 30% HBr/AcOH and treatment with Fmoc-OSu gave the *trans*-L-POPNA A monomer **7** in an overall yield of 48% from **2**. In the case of the *trans*-L-POPNA C monomer, first, N^4-benzyloxycarbonylcytosine (N^4-Cbz-cytosine)[12] was introduced to **5** to give compound **8** in 40% yield. Next, Boc and tBu protecting groups of **8** were removed with TFA to avoid deprotection of the Cbz group. The resulting free compound was treated with Fmoc-OSu to give the *trans*-L-POPNA C monomer **9** in an overall yield of 29% from **2**. In the case of the *trans*-L-POPNA G monomer, first, alkylation of 2-amino-6-chloropurine with **5** gavecompound **10** in 42% yield. Conversion of the 6-chloro group on **10** with 2-nitrophenoxy gave compound **11** in 86% yield. Next, the N^2-amino group on **11** was protected with an isobutyryl group to avoid undesirable acylation in the peptide-coupling step, to give compound **12** in 93% yield. Subsequently, removal of the 2-nitrophenoxy group on **12** with 1,1,3,3-tetramethylguanidine gave compound **13** in 65% yield.[13] Finally, removal of Boc and tBu protecting groups with 30% HBr/AcOH and treatment with Fmoc-OSu gave the *trans*-L-POPNA G monomer **14** in an overall yield of 22% from **2**.

All the final products (**4**, **7**, **9**, and **14**) were characterized by ^{1}H-NMR, ^{13}C-NMR, HRMS, and RP-HPLC (See supplementary data). Other POPNA monomers of different configurations (*cis*-L-POPNA, *trans*-D-POPNA, and *cis*-D-POPNA monomers with A, T, G, and C nucleobases) were also synthesized from corresponding starting materials and characterized in a similar manner.



Scheme 1. Synthetic routes of the *trans*-L-POPNA monomers **4**, **7**, **9**, and **14**: (i) HCOOH, DEAD, Ph_3P, THF/Toluene, rt, overnight; (ii) 25% NH_3 aq, MeOH, rt, 1 h; (iii) N^3-Benzoylthymine, DEAD, Ph_3P, THF/Toluene, rt, overnight; (iv) 30% HBr/AcOH, rt, 30 min; (v) Fmoc-OSu, $NaHCO_3$, H_2O/MeCN (1/1, v/v), rt, overnight; (vi) Ms-Cl, TEA, DCM, rt, 3 h; (vii) N^6-Benzoyladenine, K_2CO_3, 18-Crown-6, DMF, 65 ˚C, overnight; (viii) N^4-Cbz-cytosine, K_2CO_3, 18-Crown-6, DMF, 65 ˚C, overnight; (ix) TFA, rt, 30 min; (x) 2-NH_2-6-Cl-Purine, K_2CO_3, 18-Crown-6, DMF, 65 ˚C, overnight; (xi) 2-Nitrophenol, DABCO, TEA, 1,2-dichloroethane, rt, overnight; (xii) *i*PrCOCl, pyridine, rt, overnight; (xiii) 2-Nitrobenzaldoxime, 1,1,3,3-tetramethylguanidine, MeCN, rt, overnight.

Trans-L-POPNA oligomers containing four types of nucleobases were synthesized by SPPS according to previous procedures.[5] The sequences of *trans*-L-POPNA oligomers were H-TGGTGCGAA-Lys-NH_2(*trans*-L-POPNA9), Fam-Sp2-TGGTGCGAATTC-Lys-NH_2(Fam-*trans*-L-POPNA12, where Sp2 indicates a linker consisting of ethylene glycol units and Fam represents 5(6)-carboxy-fluorescein used as a fluorescent label), Fam-Sp2-TGGTGCCTC-Sp2-$(Arg)_7$-NH_2(Fam-*trans*-L-POPNA9-R7), and Fam-Sp2-CAGTTAGGGTTAG-Gly-NH_2(Fam-*trans*-L-POPNA13). Lys, Arg, and Gly indicate lysine, arginine, and glycine, respectively. The N-terminals and C-terminals of these oligomers are primary amines and primary amides, respectively. The crude oligomers were purified by preparative HPLC, and the purified oligomers were identified by MALDI-TOF Mass (*trans*-L-POPNA9, calcd. $[M + H]^+$ = 2635.13, obsd. $[M + H]^+$ = 2634.26; Fam-*trans*-L-POPNA12, calcd. $[M + H]^+$ = 3918.57, obsd. $[M + H]^+$ = 3917.95; Fam-*trans*-L-POPNA9-R7, calcd. $[M + H]^+$ = 4174.91, obsd. $[M + H]^+$ = 4175.50; Fam-*trans*-L-POPNA13, calcd. $[M + H]^+$ = 4163.63, obsd. $[M + H]^+$ = 4163.26).[14]

2.2. Hybrid formation of POPNA oligomers with DNAs

Melting curves of *trans*-L-POPNA9 in equimolar mixtures with DNA (5′-TTCGXACCA-3′, X = A, T, G, and C) are shown in Figure 2. In the case of the completely complementary DNA (X = C), the sigmoidal melting curve was observed having a melting temperature (T_m) of 22.2 ˚C. The T_m's of the hybrids of *trans*-L-POPNA9 with DNA sequences having a single-mismatched, where X = A, T, and G, were 21.4, 21.0, and 20.3 ˚C, respectively. These results indicate that *trans*-L-POPNA9, having a sequence that contains the four types of nucleobases, forms moderate sequence-specific hybrids with DNAs.

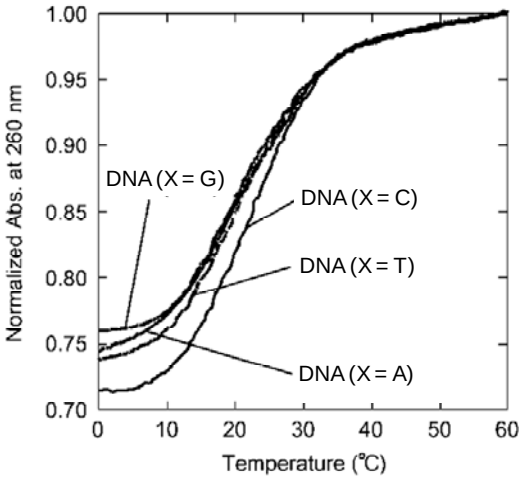


Figure 2. Temperature dependences of absorption intensities at 260 nm for equimolar mixtures of *trans*-L-POPNA9 (*N*′-TGGTGCGAA-*C*′)/DNA (5′-TTCGXACCA-3′, X = A, T, G, and C) in 100 mM NaCl, 10 mM NaH_2PO_4, and 0.1 mM EDTA, with pH 7.0. [*trans*-L-POPNA9] = [DNA] = 2.5 μM. The melting curves were recorded by heating the solution at 0.5 ˚C/0.5 min. The observed absorbance has been normalized at 60 ˚C.

To examine the effect on the configuration of POPNAs when hybridized with DNAs, we synthesized13-mer POPNA oligomers of different configurations (*trans*-L POPNA13, *cis*-L POPNA13, *trans*-D-POPNA13, and *cis*-D-POPNA13; with a sequence of H-CAGTTAGGGTTAG-NH_2.) and we estimated the melting temperatures of the oligomers-DNAs hybrids from their respective melting curves. Melting temperatures of these hybrids are listed in Table 1. Of these oligomers, *trans*-D-POPNA13 formed the most stable hybrid with the fully complementary DNA (T_m = 41.2 ˚C). *Trans*-L-POPNA13, *cis*-L-POPNA13 and *cis*-D-POPNA13 showed lower hybrid stabilities with the fully complementary DNA than *trans*-D-POPNA13. All POPNA oligomers formed moderate sequence-specific hybrids with DNAs. Successful hybridizations of all the stereoisomers of POPNA are explained in terms of their flexible main chains that contain ether linkages. However, the higher stability of the hybrid between *trans*-D-POPNA and DNA, than those of the other oligomers/DNA hybrids, is a consequence of its restricted main chain, which contains pyrrolidine rings. Moreover, hybrids of POPNA oligomers with DNAs are preferentially formed with antiparallel orientations, especially *trans*-L-POPNA13 and *cis*-L-POPNA13 (32.9 ˚C and 33.6 ˚C for antiparallel orientation and

28.6 ˚C and 27.3 ˚C for parallel orientation, respectively). This result also suggests that configurations of POPNA have a tendency to form a single, stable hybrid with DNA.

Table 1. Melting temperatures (T_m) of hybrids of 13-mer POPNAs (*N*'-CAGTTAGGGTTAG-*C*') with DNAs (5'-CTAACCXTAACTG-3', X = A, T, C, and G).

POPNA	DNA (5′-CTAACCXTAACTG-3′)[a]				
	Antiparallel orientation				Parallel orientation
	X = C (full-matched)	A	G	T	C
trans-L	32.9	29.8	30.2	29.0	28.6 (4.3)[b]
trans-D	41.2	37.8	37.4	35.7	39.9 (1.4)
cis-L	33.6	30.2	28.6	27.8	27.3 (6.3)
cis-D	32.5	28.9	27.2	26.3	30.8 (1.6)

[a]Measurement conditions of melting curves of these mixtures are the same as those of Figure 1.

[b]Values in parentheses show a difference of T_m (antiparallel orientation) for T_m (parallel orientation).

2.3. Transport of POPNA oligomers into cytoplasm

We examined the internalization of the POPNA oligomer into Chinese hamster ovary (CHO) cells with confocal laser scanning microscopy. First, the CHO cells were cultured in the presence of 10 μM Fam-*trans*-L-POPNA12. However, no fluorescence was observed with the CHO cells. Unfortunately, the POPNA oligomer did not internalize into the cells by themselves. In the case of the *trans*-L-POPNA oligomer conjugated with a CPP (Fam-*trans*-L-POPNA9-R7), the fluorescence from Fam was observed inside the cells, as shown in Figure 3. However, this image indicates that the fluorescence is mostly confined in small vesicular compartments. These endosomes were observed even after 6 h of incubation, without a decrease in fluorescence. These results suggest that the POPNA oligomer conjugated with a CPP internalized inside CHO cells by an endocytosis mechanism and that they remained inside the endosomes.

To release the POPNA oligomer from the endosomes, we synthesized an HA2 (Tmr-HA2-R7, a peptide that disrupts endosomes)[7] labeled with a 5(6)-tetramethylrhodamine fluorophore at the N-terminal and conjugated with R7 at the C-terminal. The CHO cells were incubated with the mixture of Fam-*trans*-L-POPNA13 and Tmr-HA2-R7. The fluorescence from the CHO cells, after incubation with Fam-*trans*-L-POPNA13, in the absence and presence of Tmr-HA2-R7 was measured. Incubation with only Fam-*trans*-L-POPNA13, showed no internalization of the oligomer. On the other hand, Fam-*trans*-L-POPNA13 was successfully internalized inside CHO cells in the presence of Tmr-HA2-R7 (Figure 4), and more importantly, both the red (Tmr) and green (Fam) fluorescence were detected throughout the whole cell. This indicates that the POPNA oligomers were taken up into the cells with the Tmr-HA2-R7 and released into the cytoplasm with the disruption of the endosomes by the HA2 peptide within 1 h.

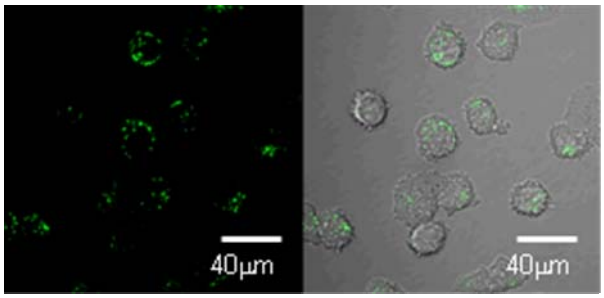


Figure 3. Confocal microscopy images of CHO cells cultured for 2 h at 37 ˚C in the presence of 1.0 μM Fam-*trans*-L-POPNA9-R7. The left image shows the green fluorescence image from Fam-fluorescence. The right image is a superimposed image of the phase contrast and the fluorescence images.

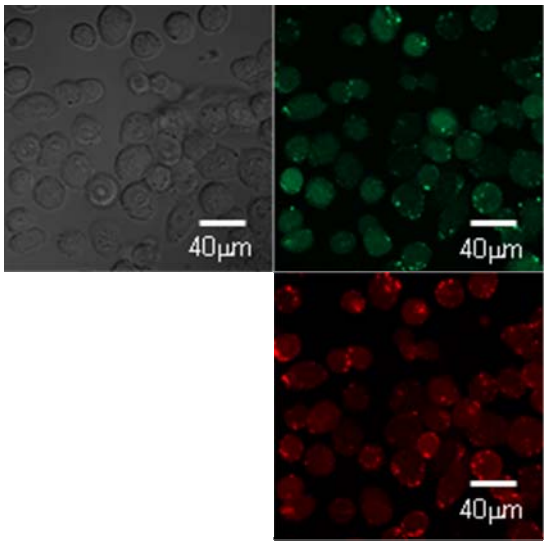


Figure 4. Confocal microscopy images of CHO cells cultured for 1 h at 37 ˚C in the presence of Fam-*trans*-L-POPNA13 and Tmr-HA2-R7 (Tmr-Sp2-GLFEAIEGFIENGWEGMIDGWYG-Sp2-RRRRRRR-NH_2). [Fam-*trans*-L-POPNA13] = [Tmr-HA2-R7] = 10 μM.[15] The top left image shows the phase contrast image of the optical section at the middle of cells. The top right image shows the green fluorescence image from Fam groups. The bottom right image shows the red fluorescence image from Tmr groups.

3. Conclusions

In summary, we synthesized sixteen POPNA monomers with four types of nucleobases on four stereoisomers of the pyrrolidine rings. POPNA oligomers of a mixed sequence of four types of nucleobases were also synthesized. These POPNA oligomers were successfully hybridized with DNAs. The configurations of POPNA oligomers affect the hybridization. The POPNA oligomers were readily taken up inside the cytoplasm of CHO cells when mixed with a HA2-CPP conjugate. The antisense effect of the four types of stereoisomers of the POPNA oligomers, in combination with the HA2-CPPconjugate is now under investigation.

4. Experimental

4.1. General

Diethyl azodicarboxylate (DEAD), triphenylphosphine (Ph_3P), methanesulfonylchloride (Ms-Cl), 25% aqueous ammonia, triethylamine (TEA), N^6-benzoyladenine, 18-crown-6, 2-amino-6-chloropurine, 2-nitrophenol, 2-nitrobenzaldoxime, K_2CO_3,

$NaHCO_3$, $KHSO_4$, $MgSO_4$, pyridine, and other solvents were purchased from Wako Chemicals (Tokyo, Japan). Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, 30% hydrogen bromide in acetic acid (HBr/AcOH), trifluoroacetic acid (TFA) and 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu) were purchased from Watanabe Chemicals (Hiroshima, Japan). Formic acid (HCOOH) and Fmoc-NH-PEG-COOH(Sp2) were purchased from Merck (NJ, USA). 1,4-Diazabicyclo[2.2.2]octane (DABCO), isobutyrylchloride (*i*PrCOCl), 1,1,3,3-tetramethylguanidine, 6-[fluorescein-5(6)-carboxamido]hexanoic acid *N*-hydroxysuccinimide ester (Fam) and 6-carboxy-tetramethylrhodamine *N*-succinimidyl ester (Tmr) were purchased from Sigma-Aldrich (MO, USA). DNA oligomers were purchased from Invitrogen Japan (Tokyo, Japan). These reagents were used without purification. Distilled water was used throughout the synthesis. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Mercury 300 spectrometer. High-resolution mass spectra were measured on Nihon Denshi MS 700. MALDI-TOF Mass spectra were measured on Applied Biosystems Voyager DE-Pro, and hybridization behavior was observed on a JASCO V-560 UV-vis spectrophotometer equipped with a temperature controller. Fluorescent images from confocal laser scanning microscopy were observed on Olympus FLUOVIEW FV-1000.

Key intermediates for POPNA monomers, **1** (*trans*-L configuration), *cis*-L-configurated **1**(**2**), *cis*-D-configurated **1**, and *trans*-D-configurated **1**were synthesized according to procedures that were previously reported.[5b]

4.2. Synthesis of POPNA T monomers

*4.2.1. (2S,4R)-4-(3-Benzoyl-5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-2-tert-butoxycarbonylmethoxymethyl-pyrrolidine-1-carboxylic acid tert-butyl ester (**3**)*

2 (3.51 g, 10.6 mmol), Ph_3P (4.17 g, 15.9 mmol), and N^3-benzoylthymine (2.39 g, 10.6 mmol) were added to tetrahydrofuran (THF)(10 mL). To this mixture was added DEAD (2.49 mL, 15.9 mmol) under argon atmosphere at –15˚C and then stirred at room temperature overnight. The resultant mixture was evaporated to dryness and the residue was washed several times with 50/50 ethyl acetate (AcOEt)/hexane (Hex) mixture and dried in vacuo. The residue was chromatographed on silica gel with 40/60 AcOEt/Hex mixture as eluting solvent. The title compound **3** was obtained as white powder. Yield: 17%. R_f (AcOEt/Hex = 5/5, v/v): 0.41. ^{1}H-NMR (300MHz, $CDCl_3$):δ1.5 (s+s, 18H, Boc CH_3 and tert-butyl CH_3), 1.96 (s, 3H, thymine CH_3), 2.2-2.6 (m, 2H, C3′H_2), 3.4-4.3 (m, 7H, C5′H_2, C_α-CH_2-O-CH_2-CO and C2′H), 5.2 (m, 1H, C4′H), 7.1 (s, 1H, C6H), 7.4-7.9 (m, 5H, phenyl). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 14.3 (thymine CH_3), 28.1 and 28.4 (Boc CH_3 and tert-butyl CH_3), 32.4 (C3′), 49.6 (C5′), 55.8 (C2′), 62.3 (C4′), 68.9 (O-CH_2-CO), 72.4 (C_α-CH_2-O), 80.5 and 81.7 (Boc C and tert-butyl C), 111.5 (C5), 129.1, 130.4, 131.5, and 134.9 (phenyl), 136.3 (C6), 149.8 (C2), 154.5 (Boc amide), 161 (C4), 168.9 (tert-butyl ester and benzoyl amide).

*4.2.2. (2S,4R)-2-Carboxymethoxymethyl-4-(5-methyl-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-pyrrolidine-1-carboxylic acid 9H-fluoren-9-ylmethyl ester (**4**, trans-L-POPNA T monomer)*

3 (1.00 g, 1.83 mmol) was treated with 30% HBr/AcOH (10 mL) for 30 min at room temperature. The resultant mixture was evaporated to dryness. The residue was dissolved in 5% aqueous $NaHCO_3$ (10 mL) to adjust the pH to 8. A solution of Fmoc-OSu (0.71 g, 2.20 mmol) in acetonitrile (MeCN, 10 mL) was added to the aqueous solution with stirring under ice cooling. The reaction mixture was stirred at room temperature overnight and evaporated to dryness. The residue was dissolved in water and the aqueous layer was washed three times with diethyl ether (Et_2O, each 30 mL), acidified to pH 7 with 5% aqueous $KHSO_4$, and extracted three times with AcOEt. The AcOEt layer was washed with water and brine, then, dried over $MgSO_4$. Filtration followed by solvent evaporation gave the title compound **4**. **4** was obtained as a white powder. **4** was purified by preparatory RP-HPLC (C18 column) before using to SPPS. Yield: 60%. ^{1}H-NMR (300MHz, $CDCl_3$):δ1.7 and 1.8 (s+s, 3H, thymine CH_3), 2.0-2.4 (m, 2H, C3′H_2), 3.0-4.2 (m, 7H, C5′H_2, C_α-CH_2-O-CH_2-CO, and Fmoc C*H*), 4.3 (m, 2H, Fmoc CH_2), 4.4-4.7 (m, 1H, C2′*H*), 5.0-5.3 (m, 1H, C4′*H*), 7.3-7.9 (m, 8H, Fmoc aromatic ring), 7.6 (s, 1H, C6*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 12.5 (thymine CH_3), 32.4 and 33.4 (C3′), 47.2 (Fmoc *C*H), 49.6 (C5′), 53.0 and 54.0 (C4′), 55.9 and 56.4 (C2′), 66.6 (Fmoc CH_2), 68.1 (O-CH_2-CO), 71.8 (C_α-CH_2-O), 111.9 (C5), 119.9, 124.9, 127.0, 127.7, 141.3, and 143.8 (Fmoc aromatic ring), 136.6 (C6), 151.3 (C2), 154.5 (Fmoc amide), 164.2 (C4), 173.6 (COOH). HRMS (found/calculated): 506.1953/506.1849 $[M+H]^+$. IR (film, $CHCl_3$, cm^{-1}): 3067, 2957, 2895, 1697, 1522, 1508, 1474, 1451, 1419, 1356, 1339, 1267, 1140. Synthesis of *cis*-L-configuration of **4** was also performed in a similar manner to that described above. Yield: 64%. ^{1}H-NMR (300MHz, $CDCl_3$):δ1.9 (m, 3H, thymine CH_3), 2.1-2.7 (m, 2H, C3′H_2), 2.9-4.8 (m, 6H, C_α-CH_2-O-CH_2-CO, Fmoc C*H*, and C2′*H*), 4.2 (m, 2H, Fmoc CH_2), 5.0-5.3 (m, 2H, C5′H_2), 5.6 (m, 1H, C4′*H*), 7.2-7.8 (m, 9H, Fmoc aromatic ring and C6*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 12.3 (thymine CH_3), 34.2 (C3′), 47.2 (Fmoc *C*H), 49.6 (C5′), 51.5 (C4′), 55.7 (C2′), 66.7 (Fmoc CH_2), 67.9 (O-CH_2-CO), 71.8 (C_α-CH_2-O), 111.4 (C5), 119.9, 125.0, 127.1, 127.7, 141.2, and 143.8 (Fmoc aromatic ring), 137.2 (C6), 151.2 (C2), 154.2 (Fmoc amide), 164.2 (C4), 172.5 (COOH). HRMS (found/calculated): 506.1914/506.1849 $[M + H]^+$. IR (film, $CHCl_3$, cm^{-1}): 3066, 2959, 2930, 2901, 1697, 1522, 1508, 1474, 1452, 1424, 1339, 1273, 1138. Synthesis of *trans*-D-configuration of **4** and *cis*-D-configuration of **4** were performed according to aforementioned protocols and these spectroscopic data were identical with **4** and *cis*-L-configuration of **4**, respectively. *Trans*-D-configuration of **4**; Yield: 36%. HRMS (found/calculated): 506.1931/506.1849 $[M+H]^+$. *Cis*-D-configuration of **4**; Yield: 40%. HRMS (found/calculated): 506.1946/506.1849 $[M + H]^+$.

4.3. Synthesis of POPNA A monomers

*4.3.1. (2S,4S)-2-tert-Butoxycarbonylmethoxymethyl-4-methane-sulfonyloxy-pyrrolidine-1-carboxylic acid tert-butyl ester (**5**)*

Ms-Cl (2.5 g, 21.6 mmol) and TEA (2.2 g, 21.6 mmol) were added to a solution of **2** (5.5 g, 16.6 mmol) in dichloromethane (DCM, 17 mL) under ice cooling, and the mixture was stirred for 3 h at room temperature. The mixture was evaporated to dryness. The residue was dissolved in water (50 mL) and extracted three times with AcOEt (each 50 mL). The AcOEt layer was washed with water (50 mL) and brine (50 mL), then, dried over $MgSO_4$. Filtration followed by solvent evaporation gave crude viscous oil. The crude oil was chromatographed on silica gel with 30/70 AcOEt/Hex mixture as eluting solvent. The title compound **5** was obtained as a colorless viscous oil. Yield: 93%. ^{1}H-NMR (300MHz, $CDCl_3$):δ1.5 (s+s, 18H, Boc CH_3 and *tert*-butyl CH_3), 2.2-2.6 (m, 2H, C3′H_2), 3.1 (s, 3H, mesyl CH_3), 3.4-4.2 (m, 7H, C2′*H*, C5′H_2 and C_α-CH_2-O-CH_2-CO), 5.2 (m, 1H, C4′*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 28.1 and 28.4 (Boc CH_3 and *tert*-butyl CH_3), 34.8 (C3′), 38.7 (mesyl CH_3), 53.1 (C5′), 55.5 (C2′), 68.8 (O-CH_2-CO), 71.4 (C_α-CH_2-O), 79.1 (C4′), 80.3 and 81.5 (Boc *C* and *tert*-butyl *C*), 154.0 (Boc amide), 169.5 (*tert*-butyl ester).

*4.3.2(2S,4R)-4-(6-Benzoylamino-purin-9-yl)-2-tert-butoxycarbonylmethoxymethyl-pyrrolidine-1-carboxylic acid tert-butyl ester (**6**)*

5 (2.5 g, 6.1 mmol), N^6-benzoyladeninne (2.9 g, 12 mmol), 18-crown-6-ether (1.0 g, 3.8 mmol), and K_2CO_3 (1.6 g, 12 mmol) were added to *N*, *N*′-dimethylformamide (DMF, 10 mL). The mixture was stirred at 60˚C overnight. The mixture was evaporated to dryness. The residue was dissolved in water (50 mL) and extracted three times with AcOEt (each 50 mL). The AcOEt layer was washed with water (50 mL) and brine (50 mL), then, dried over $MgSO_4$. Filtration followed by solvent evaporation gave crude yellow powder. The crude compound was chromatographed on silica gel with 90/10 AcOEt/Hex mixture as eluting solvent. The title compound **6** was obtained as a white powder. Yield: 41%. R_f (AcOEt/Hex = 7/3 (v/v)): 0.13. ^{1}H-NMR (300MHz, $CDCl_3$):δ1.44 and 1.48 (s+s, 18H, Boc CH_3 and *tert*-butyl CH_3), 2.6-2.8 (m, 2H, C3′H_2), 3.6-4.4 (m, 7H, C2′*H*, C5′H_2 and C_α-CH_2-O-CH_2-CO), 5.42 (m, 1H, C4′*H*), 7.4-8.0 (m, 5H, phenyl), 8.2 (m, 1H, benzoyl amide), 8.77 (s, 1H, C2*H*), 9.23 (s, 1H, C8*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 28.1 and 28.4 (Boc *C*H$_3$ and *tert*-butyl *C*H$_3$), 33.6 (C3′), 51 (C5′), 53.7 (C4′), 55.9 (C2′), 69.0 (O-*C*H$_2$-CO), 71.9 (C_α-*C*H$_2$-O), 80.3 and 81.7 (Boc *C* and *tert*-butyl *C*), 123 (C5), 127.8, 128.8, 132.7, and 133.6 (phenyl), 129 (C6), 141.3 (C8), 149.5 (C2), 152.4 (C4), 153.9 (Boc amide), 164.7 (benzoyl amide), 169.4 (*tert*-butyl ester).

*4.3.3(2S,4R)-4-(6-Benzoylamino-purin-9-yl)-2-carboxymethoxymethyl-pyrrolidine-1-carboxylic acid 9H-fluoren-9-ylmethyl ester (**7**, trans-L-POPNA A monomer)*

6 (1.4 g, 2.5 mmol) was treated with 30% HBr/AcOH (10 mL) for 30 min at room temperature. The resultant mixture was evaporated to dryness. The residue was dissolved in 5% aqueous $NaHCO_3$ (50 mL) to adjust the pH to 8. A solution of Fmoc-OSu (0.94 g, 2.8 mmol) in MeCN (50 mL) was added to the aqueous solution with stirring under ice cooling. The reaction mixture was stirred at room temperature overnight and evaporated to dryness. The residue was dissolved in water and the aqueous layer was washed three times with Et_2O (each 30 mL), acidified to pH 7 with 5% aqueous $KHSO_4$, and the resultant precipitate was collected by filtration. The residue was washed several times with water and dried *in vacuo*. **7** was obtained as a white powder. **7** was purified by preparatory RP-HPLC (C18 column) before using to SPPS. Yield: 77%.^{1}H-NMR (300MHz, $CDCl_3$):δ2.4-3.2 (m, 3H, C3′H_2 and C2′*H*), 3.5-4.8 (m, 9H, Fmoc C*H*, C5′H_2, C_α-CH_2-O-CH_2-CO and Fmoc CH_2), 5.48 and 5.67 (m+m, 1H, C4′*H*, rotamer), 7.2-8.2 (m, 13H, Fmoc aromatic ring and phenyl), 8.19 and 8.29 (s+s, 1H, C8*H*, rotamer), 8.72 and 8.80 (s+s, 1H, C2*H*, rotamer), 10.52 (br, 1H, COO*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 33.6 (C3′), 47.2 (Fmoc *C*H), 50.5 (C5′), 54.2 (C4′), 56.0 and 56.6 (C2′), 67.4 (Fmoc *C*H$_2$), 68.0 (O-*C*H$_2$-CO), 71.8 (C_α-*C*H$_2$-O), 119.9, 124.9, 127.0, 127.7, 141.2, and 143.7 (Fmoc aromatic ring), 127.2, 128.4, 132.8, and 133.0 (phenyl), 128.6 (C5), 142.4 (C8), 149.6 (C4), 151.5 (C6), 152.2 (C2), 154.2 (Fmoc amide), 165.4 (benzoyl amide), 173.5 (*C*OOH). HRMS (found/calculated): 619.2282/619.2227 $[M + H]^+$. IR (film, $CHCl_3$, cm^{-1}): 3069, 2895, 1701, 1611, 1518, 1456, 1420, 1339, 1242, 1138. Synthesis of *cis*-L-configuration of **7** was also performed in an identical manner to that described above. Yield: 89%. ^{1}H-NMR (300MHz, $CDCl_3$):δ2.3-3.2 (m, 3H, C3′H_2 and C2′*H*), 3.2-5.4 (m, 10H, Fmoc C*H*, C5′H_2, C_α-CH_2-O-CH_2-CO, Fmoc CH_2 and C4′*H*), 7.2-7.8 (m, 13H, Fmoc aromatic ring and phenyl), 7.96 (br, 1H, C8*H*), 8.76 and 8.86 (br+br, 1H, C2*H*, rotamer), 10.85 (br, 1H, COO*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 32.9 (C3′), 47.2 (Fmoc *C*H), 51.5 (C5′), 52 (C4′), 55.8 (C2′), 67.2 (Fmoc *C*H$_2$), 67.9 (O-*C*H$_2$-CO), 71.6 (C_α-*C*H$_2$-O), 119.9, 124.7, 127.1, 127.7, 141.3, and 143.8 (Fmoc aromatic ring), 128.5 (C5), 127.4, 128.2, 132.7, and 133.1 (phenyl), 142.5 (C8), 149.2 (C4), 151 (C6), 151.9 (C2), 154.2 (Fmoc amide), 165.5 (benzoyl amide), 172.9 (*C*OOH). HRMS (found/calculated): 619.2332/619.2227 $[M+H]^+$. IR (film, $CHCl_3$, cm^{-1}): 3069, 2895, 1699, 1611, 1522, 1452, 1420, 1338, 1246, 1140. Synthesis of *trans*-D-configuration of **7** and *cis*-D-configuration of **7** were performed according to aforementioned protocols and these spectroscopic data were identical with **7** and *cis*-L-configuration of **7**, respectively. *Trans*-D-configuration of **7**; Yield: 41%. HRMS (found/calculated): 619.2332/619.2227 $[M + H]^+$. *Cis*-D-configuration of **7**; Yield: 9%. HRMS (found/calculated): 619.2312/619.2227 $[M+H]^+$.

4.4. Synthesis of POPNA C monomers

*4.4.1. (2S,4R)-4-(4-Benzyloxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-2-tert-butoxycarbonylmethoxymethyl-pyrrolidine-1-carboxylic acid tert-butyl ester (**8**)*

5 (2.00 g, 4.88 mmol), N^4-Cbz-cytosine (2.99 g, 12.2 mmol), 18-crown-6 (3.22 g, 12.2 mmol) and K_2CO_3 (1.68 g, 12.2 mmol) were added to DMF (50 mL) and stirred at 65 ˚C overnight. The mixture was filtered and evaporated dryness. The crude oil was chromatographed on silica gel with 70/30 AcOEt/Hex mixture as eluting solvent. The title compound **8** was obtained as a colorless viscous oil. Yield: 40%.^{1}H-NMR (300MHz, $CDCl_3$):δ1.44 and 1.47 (s+s, 18H, Boc CH_3 and *tert*-butyl CH_3), 2.35 and 2.55 (m+m, 2H, C3′H_2), 3.4-4.3 (m, 7H, C5′H_2, C_α-CH_2-O-CH_2-CO, and C2′*H*), 5.21 (s, 2H, Cbz CH_2), 5.28 (m, 1H, C4′*H*), 7.1-7.9 (m, 8H, C6*H*, phenyl, C5*H*, and Cbz ester). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 28.1 and 28.4 (Boc *C*H$_3$ and *tert*-butyl *C*H$_3$), 32.3 and 34.2 (C3′), 50 (C5′), 51.1 (C4′), 55.6 (C2′), 67.9 (Cbz *C*H$_2$), 68.9 (O-*C*H$_2$-CO), 71.5 and 72.2 (C_α-*C*H$_2$-O), 80.2 and 81.6 (Boc *C* and *tert*-butyl *C*), 95.2 (C5), 128.3, 128.6, 131, and 134.9 (phenyl), 145.0 (C6), 152.2 (Cbz ester), 153.9 (Boc amide), 155.4 (C2), 161.7 (C4), 169.3 (*tert*-butyl ester).

*4.4.2. (2S,4R)-4-(4-Benzyloxycarbonylamino-2-oxo-2H-pyrimidin-1-yl)-2-carboxymethoxymethyl-pyrrolidine-1-carboxylic acid 9H-fluoren-9-ylmethyl ester (**9**, trans-L-POPNA C monomer)*

8 (0.50 g, 0.89 mmol) was treated with TFA(10mL) for 30 min at room temperature. The resultant mixture was evaporated to dryness. The residue was dissolved in 5% aqueous $NaHCO_3$ (50 mL) to adjust the pH to 8. A solution of Fmoc-OSu (0.33 g, 0.98 mmol) in MeCN (50 mL) was added to the aqueous solution with stirring under ice cooling. The reaction mixture was stirred at room temperature overnight and evaporated to dryness. The residue was dissolved in water and the aqueous layer was washed three times with Et_2O (each 30 mL), acidified to pH 3 with 5% aqueous $KHSO_4$, and extracted three times with AcOEt (each 50 mL). The AcOEt layer was washed with water (50 mL) and brine (50 mL) and dried over $MgSO_4$. Filtration followed by solvent evaporation gave the title compound **9**. **9** was obtained as a white powder. **9** was purified by preparatory RP-HPLC (C18 column) before using to SPPS. Yield: 54%. ^{1}H-NMR (300MHz, $CDCl_3$):δ2.1-3.2 (m, 3H, C3′H_2 and C2′*H*), 3.4-4.8 (m, 9H, Fmoc C*H*, C5′H_2, C_α-CH_2-O-CH_2-CO, and Fmoc CH_2), 5.22 (s, 2H, Cbz CH_2), 5.30 (m, 1H, C4′*H*), 7.2-7.8 (m, 15H, C6*H*, Fmoc aromatic ring, phenyl, and C5*H*) , 9.25 (br, 1H, COO*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 32.3 and 33.4 (C3′), 47.2 (Fmoc *C*H), 50.2 (C5′), 56.2 (C4′), 57.1 (C2′), 66.4 (Cbz *C*H$_2$), 67.2 (Fmoc *C*H$_2$), 67.8 (O-*C*H$_2$-CO), 71.4 (C_α-*C*H$_2$-O), 96.0 (C5), 119.9, 124.8, 127.1, 127.7, 141.2, and 143.7 (Fmoc aromatic ring), 124.4, 128.1, 128.5, and 135.0 (phenyl), 145.5 (C6), 152.8 (Cbz ester), 154.3 (Fmoc amide), 154.9 (C2), 162.2 (C4), 172.8 (*C*OOH). HRMS (found/calculated): 625.2314/625.2220 $[M+H]^+$.

Synthesis of *cis*-L-configuration of **9** was also performed in an identical manner to that described above. Yield: 10% at 2 steps. ^{1}H-NMR (300MHz, $CDCl_3$):δ1.9-3.2 (m, 4H, C3′H_2 and C5′H_2), 3.4-4.8 (m, 8H, C_α-CH_2-O-CH_2-CO, Fmoc C*H*, Fmoc CH_2 and C2′*H*), 5.2-5.5 (m, 3H, C4′*H* and Cbz CH_2), 7.1-8.8 (m, 15H, C6*H*, Fmoc aromatic ring, phenyl, and C5*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 33.2 (C3′), 47.4 (Fmoc *C*H), 51.5 (C5′), 52 (C4′), 55.8 (C2′), 67.1 (Cbz *C*H_2), 68.0 (Fmoc *C*H_2), 68.4 (O-*C*H_2-CO), 72.8 (C_α-*C*H_2-O), 96.3 (C5), 120.0, 124.8, 126.9, 127.1, 141.3, and 143.8 (Fmoc aromatic ring), 124, 127.8, 128.3, and 134.8 (phenyl), 148.6 (C6), 153.0 (Cbz ester), 154.0 (Fmoc amide), 154 (C2), 162.0 (C4), 175.9 (*C*OOH). HRMS (found/calculated): 625.2305/625.2220 $[M + H]^+$. IR (film, $CHCl_3$, cm^{-1}): 1751, 1697, 1624, 1558, 1508, 1452, 1429, 1400, 1354, 1253, 1140, 1124, 1071, 1005. Synthesis of *trans*-D-configuration of **9** and *cis*-D-configuration of **9** were performed according to aforementioned protocols and these spectroscopic data were identical with **9** and *cis*-L-configuration of **9**, respectively. *Trans*-D-configuration of **9**; Yield: 5% at 2 steps. HRMS (found/calculated): 625.2323/625.2220 $[M + H]^+$. *Cis*-D-configuration of **9**; Yield: 33% HRMS (found/calculated): 625.2326/625.2220 $[M + H]^+$. IR (film, $CHCl_3$, cm^{-1}): 1748, 1701, 1654, 1628, 1558, 1521, 1450, 1420, 1352, 1331, 1142, 1103, 1069, 993, 974.

4.5. Synthesis of POPNA G monomers

*4.5.1. (2S,4R)-4-(2-Amino-6-chloro-purin-9-yl)-2-tert-butoxycarbonylmethoxymethyl-pyrrolidine-1-carboxylic acid tert-butyl ester (**10**)*

5 (5.0 g, 12.4 mmol), 2-amino-6-chloropurin (6.3 g, 37.1 mmol), 18-crown-6-ether (9.8 g, 37.1 mmol), and K_2CO_3 (5.1 g, 37.1 mmol) were added to DMF (50 mL) and stirred at 65 ˚C overnight. The mixture was filtered and evaporated to dryness. The mixture was added to AcOEt and the insoluble residue was removed. The crude oil was chromatographed on silica gel with 70/30AcOEt/Hex mixture as the eluting solvent. The title compound **10** was obtained as a colorless viscous oil. Yield: 36%. ^{1}H-NMR (300 MHz, $CDCl_3$):δ1.45 and 1.46 (s+s, 18H, Boc CH_3 and *tert*-butyl CH_3), 2.61 (t, 2H, C3′H_2), 3.5-4.3 (m, 7H, C2′*H*, C5′H_2 and C_α-CH_2-O-CH_2-CO), 5.17 (m, 1H, C4′*H*), 5.29 (br, 2H, NH_2), 7.75 (s, 1H, C8*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 28.1 and 28.4 (Boc *C*H_3 and *tert*-butyl *C*H_3), 33.4 and 34.6 (C3′), 50.5 and 51.2 (C5′), 52.8 and 53.5 (C4′), 55.9 (C2′), 68.9 (O-*C*H_2-CO), 71.9 (C_α-*C*H_2-O), 80.3 and 81.7 (Boc *C* and *tert*-butyl *C*), 125.5 (C5), 140.5 (C8), 151.4 (C2), 153.5 (Boc amide), 158.9 (C4), 162.5 (C6), 169.3 (*tert*-butyl ester).

*4.5.2. (2S,4R)-4-[2-Amino-6-(2-nitro-phenoxy)-purin-9-yl]-2-tert-butoxycarbonylmethoxymethyl-pyrrolidine-1-carboxylic acid tert-butyl ester (**11**).* DABCO (0.46 g, 4.15 mmol) and TEA (1.26 g, 12.45 mmol) were added to a solution of **10** (2.00 g, 4.15 mmol) in 1,2-dichloroethane (20 mL) and the mixture was stirred at room temperature overnight. The mixture was evaporated to dryness. The residue was dissolved in water (100 mL) and extracted three times with DCM (each 50 mL). The DCM layer was washed with water (100 mL) and brine (100 mL), and it was then dried over $MgSO_4$. Filtration followed by solvent evaporation gave crude viscous oil. The crude oil was chromatographed on silica gel with 70/30AcOEt/Hex mixture as the eluting solvent. The title compound **11** was obtained as a yellow crystal. Yield: 78%. ^{1}H-NMR (300 MHz, $CDCl_3$):δ1.45 and 1.47 (s+s, 18H, Boc CH_3 and *tert*-butyl CH_3), 2.62 (m, 2H, C3′H_2), 3.5-4.4 (m, 7H, C2′*H*, C5′H_2 and C_α-CH_2-O-CH_2-CO), 4.84 (br, 2H, NH_2), 5.16 (m, 1H, C4′*H*), 7.41, 7.67, and 8.09 (t+t+d, 4H, phenyl), 7.69 (s, 1H, C8*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 28.1 and 28.4 (Boc *C*H_3 and *tert*-butyl *C*H_3), 33.4 and 34.7 (C3′), 51.3 (C5′), 52.6 and 53.2 (C4′), 55.9 (C2′), 69.0 (O-*C*H_2-CO), 71.9 (C_α-*C*H_2-O), 80.2 and 81.6 (Boc *C* and *tert*-butyl *C*), 115.6 (C5), 125.3, 125.5, 126.0, 134.5, 142.5, and 145.5 (phenyl), 138.9 (C8), 155.2 and 158.5 (C2 and C6), 154.1 (Boc amide), 159.1 (C4), 169.4 (*tert*-butyl ester).

*4.5.3. (2S,4R)-2-tert-Butoxycarbonylmethoxymethyl-4-[2-isobutyrylamino-6-(2-nitro-phenoxy)-purin-9-yl]-pyrrolidine-1-carboxylic acid tert-butyl ester (**12**).* **11** (1.9 g, 3.2 mmol) was dissolved in pyridine and *i*PrCOCl (0.42 g, 3.9 mmol) was added to the solution of **11** in pyridine (20 mL) under ice cooling and the mixture was stirred at room temperature overnight. The mixture was then evaporated to dryness. The residue was added to water (100 mL) and extracted three times with AcOEt (each 100 mL). The AcOEt layer was washed with 5% aqueous citric acid (100 mL) and water (100 mL), and it was then dried over $MgSO_4$. Filtration followed by solvent evaporation gave crude viscous oil. The crude oil was chromatographed on silica gel with 70/30AcOEt/Hex mixture as the eluting solvent. The title compound **12** was obtained as yellow foam. Yield: quant. ^{1}H-NMR (300 MHz, $CDCl_3$):δ1.03 and 1.05 (d, 6H, *iso*-butyl CH_3), 1.46 and 1.48 (s+s, 18H, Boc CH_3 and *tert*-butyl CH_3), 2.6-2.8 (m, 2H, C3′H_2), 3.16 (m, 1H, *iso*-butyl C*H*), 3.6-4.3 (m, 7H, C2′*H*, C5′H_2 and C_α-CH_2-O-CH_2-CO), 5.32 (m, 1H, C4′*H*), 7.4-8.2 (m+m+d, 5H, phenyl and C8*H*), 7.92 (s, 1H, *iso*-butyl amide). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 18.8 (*iso*-butyl *C*H_3), 28.1 and 28.4 (Boc *C*H_3 and *tert*-butyl *C*H_3), 33.5 (C3′), 34.2 (*iso*-butyl *C*H), 51.3 (C5′), 53.8 (C4′), 55.9 (C2′), 68.9 (O-*C*H_2-CO), 71.9 and 72.5 (C_α-*C*H_2-O), 80.3 and 81.7 (Boc *C* and *tert*-butyl *C*), 118.0 (C5), 125.5, 125.8, 126.6, 134.8, 142.2, and 145.5 (phenyl), 141.3 (C8), 151.2 (C2), 154.2 (C6), 154 (Boc amide), 159.3 (C4), 169.3 (*tert*-butyl ester), 177.3 (*iso*-butyl amide).

*4.5.4. (2S,4R)-2-tert-Butoxycarbonylmethoxymethyl-4-(2-isobutyrylamino-6-oxo-1,6-dihydro-purin-9-yl)-pyrrolidine-1-carboxylic acid tert-butyl ester (**13**).* **12** (2.2 g, 3.3 mmol) and 2-nitrobenzaldoxime (5.5 g, 33 mmol) were dissolved in MeCN (30 mL), and 1,1,3,3-tetramethylguanidine (3.5 g, 30 mmol) was added to the solution. The mixture was stirred at room temperature overnight. The mixture was evaporated to dryness. The residue was dissolved in water (100 mL) and extracted five times with DCM (each 100 mL). The DCM layer was washed with water (100 mL) and brine (100 mL), and it was then dried over $MgSO_4$. Filtration followed by solvent evaporation gave a crude viscous oil. The crude oil was chromatographed on silica gel with 95/5AcOEt/MeOH mixture as the eluting solvent. The title compound **13** was obtained as a white powder. Yield: 50%. ^{1}H-NMR (300 MHz, $CDCl_3$):δ1.22 and 1.24 (d, 6H, *iso*-butyl CH_3), 1.42 and 1.45 (s+s, 18H, Boc CH_3 and *tert*-butyl CH_3), 2.4-2.8 (m, 3H, C3′H_2 and *iso*-butyl C*H*), 3.5-4.3 (m, 7H, C2′*H*, C5′H_2 and C_α-CH_2-O-CH_2-CO), 5.02 (m, 1H, C4′*H*), 7.61 (s, 1H, C8*H*), 9.37 (br, 1H, *iso*-butyl amide), 12.08 (br, 1H, C1*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 18.9 and 19.0 (*iso*-butyl *C*H_3), 28.1 and 28.4 (Boc *C*H_3 and *tert*-butyl *C*H_3), 34.0 (C3′), 36.3 (*iso*-butyl *C*H), 50.9 (C5′), 52.1 (C4′), 56.0 (C2′), 68.9 (O-*C*H_2-CO), 71.9 (C_α-*C*H_2-O), 80.3 and 81.8 (Boc *C* and *tert*-butyl *C*), 121.6 (C5), 137.2 (C8), 147.3 and 148.1 (C2 and C6), 154.3 (Boc amide), 155.7 (C4), 169.6 (*tert*-butyl ester), 178.8 (*iso*-butyl amide).

*4.5.5. (2S,4R)-2-Carboxymethoxymethyl-4-(2-isobutyrylamino-6-oxo-1,6-dihydro-purin-9-yl)-pyrrolidine-1-carboxylic acid 9H-fluoren-9-ylmethyl ester (**14**, trans-L-POPNA G monomer).* **13** (0.9 g, 1.6 mmol) was treated with 30% HBr/AcOH (10 mL) for 30 min at room temperature. The resultant mixture was evaporated to dryness. The residue was dissolved in 5% aqueous $NaHCO_3$ to adjust the pH to 8 (30 mL). A solution of Fmoc-OSu

(0.6 g, 1.8 mmol) in MeCN (30 mL) was added to the aqueous solution with stirring under ice cooling. The reaction mixture was stirred at room temperature overnight and evaporated to dryness. The residue was dissolved in water and the aqueous layer was washed three times with Et_2O (each 30 mL) and acidified to pH 7 with 5% aqueous $KHSO_4$; the resultant precipitate was collected by filtration. The residue was washed several times with water and dried *in vacuo*. **14** was obtained as a white powder. **14** was purified by preparatory RP-HPLC (C18 column) before using SPPS. Yield: 40%. ^{1}H-NMR (300 MHz, $CDCl_3$):δ1.20 and 1.22 (d, 6H, *iso*-butyl CH_3), 2.2-2.8 (m, 3H, *iso*-butyl C*H* and C3′H_2), 3.1 (m, 1H, C2′*H*), 3.3-4.7 (m, 9H, Fmoc C*H*, C5′H_2, C_α-CH_2-O-CH_2-CO, and Fmoc CH_2), 5.0-5.3 (m, 1H, C4′*H*), 7.1-7.9 (m, 9H, Fmoc aromatic ring and C8*H*), 8.67 (d, 1H, *iso*-butyl amide), 10.0-10.3 (br, 1H, COO*H*), 12.32 (br, 1H, C1*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 19.0 (*iso*-butyl CH_3), 33.7 (C3′), 36.1 (*iso*-butyl C*H*), 47.0 (Fmoc C*H*), 51.0 (C5′), 52.7 (C4′), 56.7 (C2′), 67.4 (Fmoc CH_2), 68 (O-CH_2-CO), 71.6 (C_α-CH_2-O), 119.9, 124.8, 126.9, 127.7, 141.1, and 143.6 (Fmoc aromatic ring), 124 (C5), 138.4 (C8), 147.9 and 148.4 (C2 and C6), 154.3 and 154.7 (Fmoc amide), 155.5 (C4), 172.7 (*C*OOH), 179.7 (*iso*-butyl amide). HRMS (found/calculated): 601.2391/601.2332 $[M+H]^+$. Synthesis of *cis*-L-configuration of **14** was also performed in a manner identical to that described above. Yield: 96%. R_f (AcOEt/MeOH = 9/1 (v/v)): 0.38. ^{1}H-NMR (300 MHz, $CDCl_3$):δ1.19 and 1.21 (d, 6H, *iso*-butyl CH_3), 2.3-3.0 (m, 3H, *iso*-butyl C*H* and C3′H_2), 3.1 (m, 1H, C2′*H*), 3.4-4.9 (m, 10H, Fmoc C*H*, C5′H_2, C_α-CH_2-O-CH_2-CO, Fmoc CH_2 and C4′*H*), 7.2-8.0 (m, 9H, Fmoc aromatic ring and C8*H*), 8.50 (d, 1H, *iso*-butyl amide), 9.6-9.9 (br, 1H, COO*H*), 12.19 (br, 1H, C1*H*). ^{13}C-NMR (75 MHz, $CDCl_3$):δ 18.9 (*iso*-butyl CH_3), 32.5 (C3′), 36.0 (*iso*-butyl *C*H), 47.1 (Fmoc *C*H), 50.6 (C5′), 52.4 (C4′), 56.2 (C2′), 66.6 (Fmoc *C*H_2), 68.0 (O-*C*H_2-CO), 70.8 (C_α-*C*H_2-O), 119.9, 124.9, 127.1, 127.7, 141.2, and 143.7 (Fmoc aromatic ring), 124.5 (C5), 138.5 (C8), 147.7 and 148.6 (C2 and C6), 154.2 (Fmoc amide), 155.4 (C4), 172.4 (*C*OOH), 179.6 (*iso*-butyl amide). HRMS (found/calculated): 601.2437/601.2332 $[M + H]^+$. IR (film, $CHCl_3$, cm^{-1}): 3066, 2901, 1732, 1684, 1608, 1559, 1474, 1452, 1418, 1356, 1250, 1140, 1103, 1069, 993, 974. Synthesis of the *trans*-D-configuration of **14** and *cis*-D-configuration of **14** were performed according to aforementioned protocols, and the spectroscopic data were identical with those of **14** and the *cis*-L-configuration of **14**, respectively. *Trans*-D-configuration of **14**; Yield: 36%. HRMS (found/calculated): 601.2403/601.2332 $[M + H]^+$. *Cis*-D-configuration of **14**; Yield: 50%. HRMS (found/calculated): 601.2432/601.2332 $[M + H]^+$. IR (film, $CHCl_3$, cm^{-1}): 3066, 2901, 1683, 1607, 1559, 1474, 1451, 1418, 1362, 1317, 1250, 1190, 1142, 1031, 930.

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References and notes

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14. It must be noted that the sequence of Nielsen-type PNA, H-CAGTTAGGGTTAG-Gly-NH_2, is difficult to synthesize because of three consecutive guanine units in the sequence. In the synthesis of *trans*-L-POPNA oligomers, no such restriction is encountered.
15. The CHO cells cultured for 2 h at 37 ˚C under the same conditions were subjected to an assay for cell viability by using the Cell Counting Kit-8 (Dojin). Under the above conditions, no cytotoxicity was detected.

Supplementary Material

^{1}H-NMR and ^{13}C-NMR spectra of **1-14**, HPLC chromatograms, HRMS spectra, and IR spectra of **4**, **7**, **9**, and **14**, and melting curves of mixtures of *trans*-L-POPNA13, *trans*-D-POPNA13, *cis*-L-POPNA13, *cis*-D-POPNA13, and DNAs. Supplementary data associated with this article can be found in the online version, at doi: 00.0000/j.tet.0000.00.000.